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THE BIOCHEMICAL COMPOSITION OF THE LARVAE OF TWO STRAINS OF *ARTEMIA SALINA* (L.) REARED ON TWO DIFFERENT ALGAL FOODS

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Abstract: A sequential carbohydrate, protein, and lipid method of analysis has been used to determine the biochemical composition of freshly hatched and 48-h old larvae of two strains of the brine shrimp *Artemia salina* (L.). During a 48-h starvation period the percentage of carbohydrates and lipids of freshly hatched larvae decreases whereas the ash content increases by 40–100%. When fed with dried *Scenedesmus* or dried *Spirulina* for 2 days after hatching the protein level of the larvae increases significantly and the relative increase in ash content is lower than in the case of starvation.

Amino acid analyses of the algal food and the unfed and fed larvae did not show any change except for the absence (below detection) of methionine in the starved nauplii.

The fatty acid pattern of 48-h old *Artemia* larvae is different from that of freshly hatched nauplii both in unfed and fed larvae; in the latter case it seems to be determined to a large extent by the fatty acid composition of the food.

INTRODUCTION

Although freshly hatched *Artemia salina* (L.) nauplii are the most commonly used source of food for the culture of fish and crustacean larvae (see, *e.g.*, May, 1970; Mootz & Epifanio, 1974; Schleser & Tchobanoglous, 1974), they are not a natural diet for crustaceans (Roberts, 1974) or fish (Kristenen & Hulscher-Emeis, 1972) since they are only abundant in environments with very high salinity. Nutritional deficiencies have been reported by some workers. For example, Dannevig & Hansen (1952) considered that an *Artemia* diet was not adequate for the culture of larval *Clupea*; Morris (1956) pointed out that fish larvae did not thrive as well on *Artemia* nauplii which has used up their yolk as on freshly hatched nauplii. Indeed, on the basis of the caloric content of the food, the fish larvae have to ingest 27% more second and third stage *Artemia* larvae, than first stage (Benijts *et al.*, 1976).

The geographical origin of the *Artemia* strain also seems to be very important. In 1966, Shelbourne observed "...unexpectedly heavy losses of sole larvae (*Solea solea* L.), when using *Artemia* nauplii hatched from Great Salt Lake (Utah, U.S.A.) cysts" whereas the nauplii from the San Francisco Bay (California, U.S.A.) strain

were a satisfactory food (Wickins, 1972). Various suggestions, including possible differences in biochemical composition (Little, 1969; Reeve, 1969) and the toxic concentrations of pesticides (Bookhout & Costlow, 1970), have been made to explain the poor nutritional value of brine shrimp nauplii from Great Salt Lake as compared with those from San Francisco Bay.

Wickins (1972) found that Utah nauplii can be converted into a valuable source of food for prawn larvae (*Palaemon serratus*) by feeding the larvae which had been cultured for a maximum of four days on live algae (*Isochrysis galbana*). In contrast, starvation of the freshly hatched nauplii did not improve their original poor nutritional value. No data were given on the biochemical composition of the fed and unfed *Artemia*.

The purpose of the present study is to investigate the effect of culturing both Great Salt Lake and San Francisco Bay *Artemia* nauplii for 48 h on a diet of dried algae (drum dried *Scenedesmus* sp. or spray dried *Spirulina* sp.) and to determine their biochemical composition. Both newly hatched nauplii and larvae starved for 48 h, were analysed as controls.

Material and Methods

Two series of parallel experiments were made with the *Artemia* strain from San Francisco Bay (California, U.S.A.) and that from the Great Salt Lake (Utah, U.S.A.). The cysts were incubated in filtered natural sea water (S, 30‰) at 28 °C in cylindro-conical glass tubes (Sorgeloos & Persoone, 1975). The hatched larvae were harvested and cleared from the hatching debris using an *Artemia* separator box (Persoone & Sorgeloos, 1975). Approximately 250 000 nauplii were distributed into glass aquaria containing 58 l of filtered natural sea water (S, 30‰; T, 28 °C). The aquaria were equipped with air water pumps according to the raceway technique of Mock, Neal & Salser (1973). Every hour, 20 ml of a concentrated food suspension were added to the culture tank by a peristaltic pump. The food consisted of 60 g dried algae, either spray dried *Spirulina* (Sosa Texcoco, S.A., Mexico City, Mexico) or drum dried *Scenedesmus* (Kohlenstoffbiologische Forschungsstation e.V., Dortmund, Federal Republic of Germany), per l brine of salinity 280‰. In order to reduce the particle size of the food of the appropriate size the *Scenedesmus* flakes were treated in a pearl mill. A small amount of non-toxic antifoam agent (Dow Corning, Brewing Aid No. 525, Glamorgan, U.K.) kept the food suspension from excessive foaming.

The culture period lasted 48 h. Each series consisted of: 1) starved animals; 2) animals fed with drum dried *Scenedesmus*; and 3) animals fed with spray dried *Spirulina*. The larval density was checked daily by taking three subsamples of 30 ml per raceway; the same samples were used to determine the average body length of the larvae as a criterion for larval growth. After 48 h of culturing the larvae were harvested by filtration, thoroughly washed with distilled water, frozen with liquid

nitrogen and freeze-dried. Two batches of first instar nauplii from the San Francisco Bay strain and the Great Salt Lake strain, respectively, were processed in the same way. After drying, all material was ground to a fine powder with a pestle and mortar and stored in stoppered vessels. Samples of the dried algae were ground with HCl-washed sand.

The different samples were extracted with chloroform–methanol (2:1), mixed with a Vortex mixer, and centrifuged. After the supernatant had been transferred into a small vial, the extraction procedure was repeated on the residue and the two supernatant fractions thoroughly mixed. The lipid content was determined on the supernatant and the carbohydrate and proteins on the residue.

Total lipids

The supernatant was shaken with 0.2 ml of 0.8% NaCl in 0.1 N HCl to remove water-soluble impurities (Folch *et al.*, 1957). The mixture was then allowed to separate overnight at 0 °C and the lower layer separated and dried by addition of Na_2SO_4. After filtration and evaporation at a maximum temperature of 60 °C under nitrogen, the residue was weighed on a micro-balance.

Carbohydrates and proteins

15% TCA was added to the residue which was then mixed with a Vortex mixer. After 10 min at 4 °C the suspension was centrifuged. This procedure was repeated with 5% TCA and the two supernatant fractions mixed.

Carbohydrates

The carbohydrate was determined by the method of Dubois *et al.*, 1956.

Proteins

The residue was dissolved in 1 ml 1 N NaOH by heating at 100 °C. After cooling, 9 ml distilled water were added. 0.2 ml of this 0.1 N NaOH solution was then transferred to a test tube and the protein determined by a modified Lowry method, adding a double amount of sodium potassium tartrate. The additional sodium potassium tartrate maintains the copper ions in solution during heating at 100 °C (Dorsey, pers. comm.).

Ash content

Freeze-dried samples were ignited for 4 h at 550 °C. After cooling for 30 min in a desiccator under vacuum, the ash was weighed on a micro-balance.

Fatty acid analyses

The qualitative and the quantitative fatty acid composition was determined by gas chromatography. Freeze-dried samples were esterified with 5% sulphuric acid in methanol. After 17 h, the methyl esters were extracted with petroleum ether, washed with a saturated sodium chloride solution and dried over magnesium sulphate. The organic solvent was evaporated at 30 °C under nitrogen. The gas chromatographic analysis was carried out with a Carlo Erba Fractovap 2300, equipped with an F.I.D. detector and temperature programmed from 90–170 °C at 5 °C/min. A 4 m glass column packed with 5% EGSSX on gaschrom Q (80–100 mesh) was used.

Peaks were identified from the retention times of standard esters and by plotting the log retention times against carbon numbers of the homologous series. The weight of the fatty acid methyl esters was determined as a ratio of their peak areas to that of an internal standard (pentadecanoic acid).

Amino acid analysis

Freeze-dried material was weighed in a long-necked ampoule (Gardner & Lee, 1973) and hydrolysed by 6N HCl in a sealed tube under nitrogen. Amino-acid analyses were done with a semi-automated amino acid analyzer patterned after that of Hare (1975). *o*-Phthalaldehyde was used to form fluorescent end-products with amino acids. Details on the methodology are given by Gardner (1977). It should be noted that with the analytical method used, proline, cystine, lysine, arginine, and tryptophan are not detected.

RESULTS

Growth and mortalities for the two series are summarized in Table I. The mortality was not significant in either starved or fed cultures. The first instar stage nauplii from Great Salt Lake are significantly larger than those from San Francisco Bay (Fig. 1), which corroborates the findings of D'Agostino (1965). Under the given conditions, the larvae of both strains grew faster on a *Spirulina* diet than on *Scenedesmus* cells. In the starved animals, the growth progressed little beyond the stage reached when the yolk was absorbed, although the larvae survived well.

Data on the biochemical composition of the starved animals and of the larvae fed on the two algal species are summarized in Tables II–V. From Table II it appears that during 48-h starvation at 28 °C the ash content of the San Francisco Bay larvae increased by a factor of 2 (from 8.17 to 19.97% of the body weight), whereas for Great Salt Lake larvae by only 40% (from 9.52 to 13.29%); the percentage of carbohydrates and lipids in the body decreased during starvation. When the larvae were fed, the increase in organic content surpasses the increase in inorganic content, which results in lower percentages of ash content.

TABLE I

Culture density and average length (mm) of freshly hatched, 24- and 48-h old larvae of two *Artemia* strains, starved or fed on two algal diets.

	San Francisco Bay			Great Salt Lake		
	0 h	24 h	48 h	0 h	24 h	48 h
Starved						
Density of the larvae (*N*/ml)	3.92	3.35	3.25	4.00	4.24	3.39
Average body length of larvae, mm (±S.D.)	0.53 (±0.04)	0.71 (±0.05)	0.70 (±0.09)	0.81 (±0.07)	1.09 (±0.09)	1.17 (±0.08)
Fed with *Spirulina*						
Density of the larvae (*N*/ml)	4.24	3.95	4.23	4.24	4.52	3.08
Average body length of larvae mm (±S.D.)	0.56 (±0.06)	1.03 (±0.04)	1.63 (±0.08)	0.84 (±0.08)	1.17 (±0.06)	2.10 (±0.15)
Fed with *Scenedesmus*						
Density of the larvae (*N*/ml)	3.69	3.66	3.75	3.74	–	3.44
Average body length of larvae, mm (±S.D.)	0.54 (±0.02)	0.91 (±0.05)	1.24 (±0.08)	0.70 (±0.05)	1.28 (±0.06)	1.77 (±0.09)

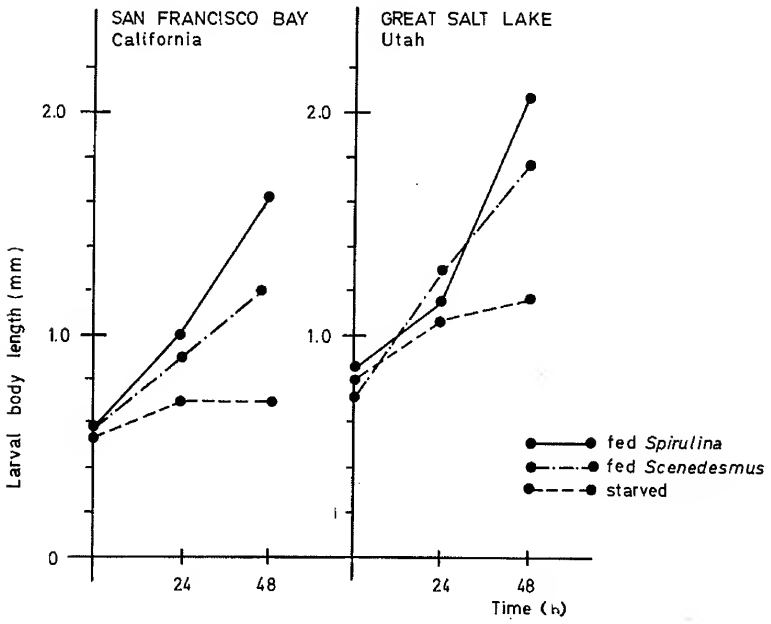

Fig. 1. Increase in body length (mm) of *Artemia* nauplii of San Francisco Bay and Great Salt Lake, starved and fed on two different diets.

TABLE II

Biochemical composition of freshly hatched nauplii of two different *Artemia* strains; 48-h old larvae, starved; 48-h old larvae, fed on two different algal diets; and the two algal diets, *viz.*, dried *Spirulina* and dried *Scenedesmus*; (1) Sosa Texcoco, S.A., Mexico City, Mexico; (2) Kohlenstoffbiologische Forschungsstation e.V., Dortmund, Federal Republic of Germany.

		% ash-free dry weight					
	Ash (%)	Water	Protein	Carbohydrates	Fibers	Lipids	Fatty acids
San Francisco Bay							
first instar nauplii	8.17		47.26	11.24		23.53	4.04
48-h old larvae,							
starved	19.97		48.88	10.37		21.95	4.01
fed *Spirulina*	10.15		61.23	12.08		25.00	4.71
fed *Scenedesmus*	8.67		59.61	10.38		28.46	5.44
Great Salt Lake							
first instar nauplii	9.52		47.24	10.54		20.84	5.97
48-h old larvae,							
starved	13.29		64.99	8.33		18.51	5.16
fed *Spirulina*	10.79		59.90	19.39		22.51	6.54
fed *Scenedesmus*	9.85		59.77	11.05		20.99	5.54
Spirulina (1)	7.97		71.76	5.62		13.14	6.43
Spirulina (1) (Durant-Chastel & Clement, 1975)	8		71.76			7.8	
Spirulina (2) (Soeder, 1976)		11	61.67	17.20		2.3	
Scenedesmus (2)	6.88		54.16	4.55		14.72	6.49
Scenedesmus (2) (Soeder, 1976)	6.10	4.9	54.65	11.18	3.11	13.15	

The pattern of amino acids (Table III) is similar in all samples. The high percentage of glutamic acid in both samples of *Artemia* fed *Scenedesmus*, reflects the high amount of this amino acid in this algal food although the high percentages of glutamic acid in *Spirulina* and of alanine and tyrosine in *Scenedesmus* are not reflected in the amino acid pattern of *Artemia* fed on these diets.

Both *Spirulina* and *Scenedesmus* are rich in fatty acids (Tables II and IV), but the pattern of the various components is different for the two algae (Table V). *Spirulina* contains 30.09 μg/mg dry wt of $C_{20:0}$. This represents 46.77% of the total fatty acids (including mono- and diphospho acids). *Spirulina* is also rich in $C_{16:0}$, $C_{16:1}$, $C_{18:2}$, and peak 12 ($C_{20:1}$). *Scenedesmus* contains 26.52 μg/mg dry wt of $C_{18:3}$, which represents 40.89% of the total fatty acids, and shows a marked concentration of $C_{16:0}$, peak 4 ($C_{17:0}$), $C_{18:1}$; peak 9 ($C_{19:0}$), $C_{18:2}$, and peak 14.

The most important saturated fatty acid in *Artemia* samples is palmitic acid which represents 16–22% of the total fatty acids. Starvation, however, lowered the per-

TABLE III

Percentage amino acid composition of 48-h old larvae of the two *Artemia* strains, starved and fed on two different algal diets; and the two algae *Spirulina* and *Scenedesmus* compared with data from Meffert (1961); [1] Kohlenstoffbiologische Forschungsstation e.V., Dortmund.

Amino acids	San Francisco Bay larvae (48 h)			Great Salt Lake larvae (48 h)					Drum-dried *Scenedesmus*[1] (Meffert, 1961)
	Starved	Fed *Spirulina*	Fed *Scenedesmus*	Starved	Fed *Spirulina*	Fed *Scenedesmus*	*Spirulina*	*Scenedesmus*	
Aspartic acid (Asp)	13.8	15.6	13.2	12.4	13.2	12.2	12.2	12.2	12.2
Threonine (Thr)	5.9	5.9	5.2	6.6	5.9	6.2	4.6	5.5	6.8
Serine (Ser)	6.4	6.0	3.9	6.7	5.7	5.4	5.0	4.0	5.0
Glutamic acid (Glu)	13.1	12.1	16.3	14.6	13.1	16.6	20.9	15.2	14.5
Glycine (Gly)	15.5	11.3	13.7	14.0	11.7	14.9	9.5	16.6	9.0
Alanine (Ala)	11.2	10.7	12.1	12.7	12.2	10.4	10.6	16.4	13.2
Valine (Val)	8.0	8.3	8.8	8.1	8.4	5.9	8.7	8.8	8.8
Methionine (Met)	0.0	2.2	0.8	0.0	2.3	2.6	2.2	0.3	2.0
Isoleucine (Ile)	6.3	6.6	6.6	6.2	5.4	5.5	5.6	4.1	4.5
Leucine (Leu)	8.9	9.9	9.8	8.2	8.9	6.2	10.2	9.9	12.2
Tyrosine (Tyr)	2.9	4.2	2.5	2.9	3.1	5.8	3.8	8.4	4.0
Phenylalanine (Phe)	5.6	4.9	4.6	4.3	5.2	6.7	4.8	4.7	5.5
Histidine (His)	2.5	2.3	2.5	3.2	4.8	1.7	1.8	1.5	2.4

TABLE IV

Fatty acid composition (μg/mg dry wt) of freshly hatched nauplii; 48-h old larvae of the two *Artemia* strains starved and fed on two algal diets; and the two algae *Spirulina* and *Scenedesmus*.

					San Francisco Bay				Great Salt Lake			
					48-h old larvae				48-h old larvae			
Peak number	Fatty acid		*Spirulina*	*Scenedesmus*	Instar I	Starved	Fed *Spirulina*	Fed *Scenedesmus*	Instar I	Starved	Fed *Spirulina*	Fed *Scenedesmus*
1	?							0.68				0.95
2	Palmitic acid	$C_{16:0}$	14.00	8.23	5.74	4.06	7.39	7.15	11.22	5.75	13.04	8.04
3	Palmitoleic acid	$C_{16:1}$	3.44	–	3.12	2.46	2.41	3.31	3.52	0.87	5.91	2.98
4	Margaric acid	$C_{17:0}$?	–	3.59	–	–	0.64	1.52	2.35	0.87	–	2.86
5		$C_{16:2}$?	–	–	0.92	0.43	1.22	0.76	0.34	0.36	1.72	0.64
6	Heptadecenoic acid	$C_{17:1}$	–	–	0.52	–	0.32	0.23	0.34	0.87	0.49	0.72
7	Stearic acid	$C_{18:0}$	0.68	–	1.56	5.18	6.20	6.32	2.47	5.68	8.48	4.30
8	Oleic acid	$C_{18:1}$	1.09	8.15	14.00	16.34	8.20	11.56	21.87	19.45	14.75	12.26
9	Nonadecanoic acid	$C_{19:0}$?	–	15.84	–	–	–	0.91	–	–	–	0.72
10	Linoleic acid	$C_{18:2}$	6.34	4.84	2.86	1.55	4.18	2.17	3.59	2.48	5.91	3.02
11	Arachidic acid	$C_{20:0}$	30.09	1.84	–	0.86	6.69	1.37	–	1.96	1.48	0.14
12	Eicosaenoic acid	$C_{20:1}$?	8.70	–	–	–	3.09	0.33	–	–	4.43	0.24
13	Linolenic acid	$C_{18:3}$	–	26.52	7.30	2.80	2.31	11.98	11.22	7.30	4.30	11.54
14	?		–	4.00	1.04	–	–	2.97	–	–	–	1.22

TABLE V

Percentage fatty acid composition of freshly hatched nauplii; 48-h old larvae of the two *Artemia* strains, starved and fed on two algal diets; the two algae *Spirulina* and *Scenedesmus* compared with some data from Wickins (1972) and Benijts *et al.* (1976).

				San Francisco Bay				Great Salt Lake						
					48-h old larvae				48-h old larvae			Wickins (1972)		Benijts *et al.* (1976)
Peak number	Fatty acid	*Spirulina*	*Scenedesmus*	Instar I	Starved	Fed *Spirulina*	Fed *Scenedesmus*	Instar I	Starved	Fed *Spirulina*	Fed *Scenedesmus*	San Francisco Bay Instar I	Great Salt Lake Instar I	San Francisco Bay Instar I
1	?						1.33				1.91			
2	$C_{16:0}$	21.26	12.69	15.49	11.98	17.33	13.95	19.71	12.67	21.55	16.20	13.56	11.75	11.4
3	$C_{16:1}$	5.35	–	8.42	7.26	5.65	6.46	6.18	1.91	9.77	6.00	8.20	4.50	5.7
4	$C_{17:0}$?	–	5.53	–	–	1.50	2.97	4.13	1.91	–	5.76	0.80	0.97	–
5	$C_{16:2}$?	–	–	2.48	1.27	2.86	1.48	0.60	0.79	2.84	1.29	2.21	2.97	–
6	$C_{17:1}$	–	–	1.40	–	0.75	0.45	0.60	1.91	0.81	1.45	1.57	2.12	–
7	$C_{18:0}$	1.06	–	4.21	15.29	14.54	12.33	4.34	12.45	14.01	8.66	5.66	4.65	5.2
8	$C_{18:1}$	1.96	12.57	37.78	48.23	19.23	22.55	38.42	42.66	24.38	24.70	29.18	23.32	32.2
9	$C_{19:0}$?	–	24.42	–	–	–	1.78	–	–	–	1.45	1.15	0.48	–
10	$C_{18:2}$	9.85	7.46	7.72	4.57	9.80	4.23	6.31	5.44	9.77	6.09	6.05	8.81	7.1
11	$C_{20:0}$	46.77	2.84	–	2.54	15.69	2.67	–	4.30	2.45	0.28	0.64	5.87	–
12	$C_{20:1}$?	13.52	–	–	–	7.25	0.64	–	–	7.32	0.48	–	–	–
13	$C_{18:3}$	–	40.89	19.70	8.26	5.42	23.37	19.71	16.01	7.11	23.25	22.27	25.23	23.9
14	?	–	6.17	2.81	–	–	5.79	–	–	–	2.46	–	–	–

centage of the former acid in the larvae to 12% (Table V). Stearic acid is almost absent in the algal foods; its percentage in the freshly hatched nauplii is relatively low compared with the 48-h larvae. Arachidic acid, although present in both series of growing larvae, does not seem to be present in the first instar from either of the geographical strains analysed. There is considerable variation in the percentages of oleic acid ($C_{18:1}$) and linolenic acid ($C_{18:3}$) in the respective *Artemia* samples. The true level of oleic acid decreased when the larvae were fed, but remained practically unchanged when unfed (Table IV). The level of linolenic acid decreased in unfed larvae as well as in larvae fed *Spirulina*. The high level of this fatty acid in larvae fed *Scenedesmus* probably only reflects the high linolenic acid content in *Scenedesmus* itself. Some typical peaks in both food sources are easily recognized in the patterns of the *Artemia* fed on them; *e.g.*, respective algal foods: the level of $C_{20:1}$ in both strains of *Artemia* fed *Spirulina* is about half of that in the alga itself and $C_{17:0}$ and the acid of peak 14 of *Scenedesmus* are assimilated much better by both *Artemia* strains than is $C_{19:0}$. On the other hand, peak 1, although absent in *Scenedesmus*, is found solely in both samples of *Artemia* larvae fed *Scenedesmus* and not in the larvae fed *Spirulina* or starved.

Discussion

Artemia salina nauplii appear to be very resistant to starvation. Under laboratory conditions at 28 °C, mortality was negligible during the 48-h experiment. The growth of the larvae was significantly faster on a *Spirulina* diet than on *Scenedesmus*, which may be explained by the higher protein content of *Spirulina* (72% compared with 55% in *Scenedesmus*). This confirms the findings of Sick (1976) who found a positive correlation between the growth rate of *Artemia* larvae and the protein content of the algal food. The percentage of protein in the larvae increases during growth, in the fed as well as in the starved animals consequent upon rapid protein synthesis in growing organisms. The smaller increase in protein in the starved larvae of the San Francisco Bay strain as compared with those of the Great Salt Lake may be due to the lower growth rate of the former nauplii. For both geographical strains, starvation of the larvae results in an increase in the ash content and a decrease in carbohydrate and lipid content.

The presence of methionine in the diet is of vital importance to the raising of sole larvae. This was concluded by Michotte (pers. comm.) from experiments with various diets including *Artemia* larvae from different geographical origin, and from the respective amino-acid patterns. Consequently the low level (below detection) of the important amino acid, methionine, in starved *Artemia* larvae of both strains is an important feature. One should, however, determine whether methionine is not partially destroyed by the hydrolysis of those proteins.

It appears (Table V) that starvation results in a relative decrease in some fatty

acids (*e.g.*, palmitic acid, linoleic acid, and linolenic acid) as others increase (*e.g.*, stearic, oleic, and arachidic acids). The reduction in content of linoleic and linolenic acids is more evident in the San Francisco Bay strain than in Great Salt Lake larvae. In contrast, the depletion of palmitic acid during starvation is striking for both strains, and corroborates the results of Culkin & Morris (1969), who found an average of 16–19% of palmitic acid in eight different marine crustaceans and a mean of 12% in the same organisms after starvation.

Oleic acid is quantitatively the most important fatty acid. Contrary to the general decrease in absolute content of most of the other fatty acids, the level of oleic acid remains practically unchanged after starvation (Table IV).

The level of stearic and arachidic acid, on the other hand, is higher in the 48-h old larvae than in the freshly hatched nauplii. In agreement with the findings of Pocock *et al.* (1971), who studied the lipid composition in the polychaete *Nereis virens*, we may conclude that in *Artemia salina* starvation significantly alters the lipid composition. Since during this period there is no nutritional input of particular fatty acids it is probable that some acids are preferentially used in metabolism, while others may be synthesized.

There are also major changes in the fatty-acid pattern during the aging process of fed animals. In *Artemia* larvae fed for 48 h, it appears that the fatty-acid pattern becomes similar for both geographical strains after feeding with *Spirulina* or *Scenedesmus*. As compared with 48-h old starved larvae, the level of palmitic acid is higher in the fed animals and the oleic acid level lower. Typical changes observed for the fatty-acid pattern in larvae fed *Spirulina* are the relatively high levels of linoleic acid, arachidic acid (not in Utah Salt Lake larvae), and the $C_{20:1}$ acid. Clearly, the very high content of these three fatty acids in the algal food is responsible for the high values in the fed *Artemia* nauplii; similarly, the relatively low content of linolenic acid in the *Artemia* larvae fed *Spirulina* may be explained by the absence of this fatty acid in the algal food. Typical changes in the fatty-acid pattern of larvae fed *Scenedesmus* are the high levels of $C_{19:0}$, linolenic acid, and peak 14. The relatively low level of arachidic acid in *Scenedesmus* is also reflected in the composition of the *Artemia* fed this alga.

The nauplii fed *Spirulina* or *Scenedesmus* partly assimilate lipids from the diet. A similarity in the fatty-acid pattern of herbivorous zooplankton and its phytoplanktonic food has been observed by many authors (see, Kayama *et al.*, 1963; Jezyck & Penicnak, 1966; Malins & Wekell, 1969, quoted by Farrington *et al.*, 1973; Ackman *et al.*, 1970; Culkin & Morris, 1969; Hinchcliffe & Riley, 1972; Bottino, 1974; Watanabe & Ackman, 1974; Sick, 1976).

From our results, it also appears that nauplii of *Artemia salina* change their fatty-acid pattern in the absence of food. That the members of higher trophic levels in the aquatic food chain can maintain a degree of control over the composition of their fatty acid is evident from the fact that Kayama *et al.* (1963) have demonstrated that fish can convert linolenic acid into eicosapentaenoic acid and docosahexaenoic

acids while Jezyck & Penicnak (1966) showed that brine shrimp can synthesize higher polyunsaturated acids, probably through the pathways of the linolenic family. Culkin & Morris (1969) have suggested that the two mono-unsaturated acids, eicosaenoic ($C_{20:1}$) and docosaenoic acid ($C_{22:1}$), present in relatively high amounts in the marine crustaceans *Sergestes corniculum*, *Gennadas valess*, and two *Acanthephyra* spp. could be formed by simple chain extention of oleic acid.

Summarizing, it is clear that the biochemical structure and subsequently its nutritional value of a planktonic organism is not only genetically determined, but is also influenced by its physiological age and by various environmental factors (Lewis, 1967; Jeffries, 1969, 1970), and not least by the ingested food. The present results combined with the work of Wickins (1972), Sick (1976), and Benijts *et al.* (1976) show that *Artemia salina* is in no way an exception to this general rule. In the case of freshly hatched instar I nauplii, we refer to a previous work in which it was shown that the fatty-acid pattern is even dependent on the environmental and nutritional conditions in which the wintering eggs were produced (Claus *et al.*, 1977).

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